Title
Development of potentiostat platform for complex voltammetric measurements and self-adjustable electrolysis

Author
Chan, Yin Shing Gary

Publication Date
2020

Peer reviewed|Thesis/dissertation

UNIVERSITY OF CALIFORNIA

Los Angeles

Development of potentiostat platform for complex voltammetric measurements and self-adjustable electrolysis

A thesis submitted in partial satisfaction of the requirements for the degree

Master of Science in Chemistry

by

Yin Shing Gary Chan

2020

ABSTRACT OF THE THESIS

Development of potentiostat platform for

complex voltammetric measurements and self-adjustable electrolysis

by

Yin Shing Gary Chan

Master of Science in Chemistry

University of California, Los Angeles, 2020

Professor Chong Liu, Chair

With rapid technological progress, electrochemical methods are being developed and improved throughout the years. Outdated models of electrochemical equipment like potentiostats are replaced with newer models with more functions and capability. Since those outdated models are programmed using programming language like C++, users can utilize the source code to program new features to the outdated potentiostats, extending its period of use. We developed methods enabling users to create new functionality of an electrochemical analytical system (Emstat3 from PalmSens BV®) using its software development platform on MATLAB® to perform complex voltammetric measurements and self-adjustable electrolysis. Complex voltammetric measurement is defined by running chronoamperometry under arbitrary potential waveform rather than the traditional constant voltage chronoamperometry or triangular potential waveform used in cyclic voltammetry. Self-adjustable electrolysis is demonstrated as a proof-of-concept via a nanowire array electrode, monitoring, and self-adjusting potentiostat parameters throughout the

experiment with the execution of a control loop. The development of arbitrary waveform allows the potential improvement of sensitivity in electroanalytical methods for detecting compounds and their redox potential, whereas the development of self-adjustable electrolysis facilitates in the self-optimization of method parameters in electrochemical measurement through a control loop mechanism.

The thesis of Yin Shing Gary Chan is approved.

Xiangfeng Duan

Richard B. Kaner

Chong Liu, Committee Chair

University of California, Los Angeles

2020

TABLE OF CONTENTS

LIST OF ABBREVAION AND SYMBOLS

Abbreviation	Full description
C.E.	Counter Electrode
C	Concentration of Analyte in Randles–Sevcik equation
c_0	Initial Concentration of the Analyte
C_D	Diffusion Layer Capacitance
C_{dl}	Double Layer Capacitance
C_H	Helmholtz Layer Capacitance
CV	Cyclic Voltammetry
D	Diffusion Constant
d	Distance of Helmholtz Layer
DO	Dissolved Oxygen
e	Elementary Charge
F	Faraday Constant
i	Complex Number
I	Current
IHL / OHL	Inner Helmholtz Layer/ Outer Helmholtz Layer
ITO	Indium Tin Oxide
j	Current Density
j_0	Exchange Current Density
j_p	Peak Current Density
k	Boltzmann constant
K_p	Proportional constant

Abbreviation	**Full description**
LSV	Linear Scan Voltammetry
n	Number of electrons
NHE	Normal Hydrogen Electrode
OCP	Open Circuit Potential
ORR	Oxygen Reduction Reaction
P Gain	Proportional Gain
PBS	Phosphate-buffered saline
PID controller	Priportional-Intergral-Derivative controller
R	Universal Gas Constant
R'	Uncompensated Resistance between R.E. and C.E.
R.E.	Reference Electrode
R_{CT}	Charge Transfer Resistance
Rs	Series Resistance between W.E. and R.E.
SHE	Standard Hydrogen Electrode
T	Temperature
t	Time
W	Wurburg Impedance
W.E.	Working Electrode
Z	Impedance
z	Charge Magnitude of Each Ion in Electrolyte
α_a	Anodic Charge Transfer Coefficient
α_c	Cathodic Charge Transfer Coefficient

Abbreviation	**Full description**
ε	Dielectric Constant
ε_0	Permittivity of Free Space
η	Overpotential
σ	Wurburg Constant
υ	Scanrate
ϕ	Inner Potential at Double Layer
ω	Frequency

LIST OF FIGURE

LIST OF TABLE

ACKNOWLEDGMENTS

I would like to thank all my members in our group, especially Shengtao Lu who assisted me in the method development of potentiostat. I would also like to thank my parents for their never-ending support and encouragement in my life. Finally, yet importantly, I would like to thank my Principal Investigator, Professor Chong Liu for this opportunity and his tremendous help in my research.

1 Introduction

Nowadays, analytical electrochemical systems are developed and improved extensively due to consumer needs in the field of research to provide more functionalities in a single system. Although building a potentiostat that fits the use from bottom-up is a viable solution, designing a potentiostat from bottom-up would require tremendous effort. Thus, software development of the existing potentiostats creates an alternative to the reinvestment of potentiostats for newer or customized functions, prolonging the service life of the existing potentiostats while catering to the needs of research. Besides, software development could enable researchers to reuse already phased-out potentiostats, creating new functionality from old infrastructure. Software development of existing commercial potentiostat if the code controlling the potentiostat were made open-source and the software development kit being available for the developers.

We developed methods enabling users to create new functionality of an electrochemical analytical system (Emstat3 from PalmSens BV®) using its software development platform. In this thesis, methods of running in arbitrary electrochemical waveform with respect to time and generating a feedback control loop for the electrochemical study is developed.

One example of the importance of achieving arbitrary electrochemical waveform testing arises from the ability to perform non-triangular waveform linear sweep voltammetry. Compton group from Oxford University has reported using a semi-circular potential sweeps could greatly amplify the peak current of the targeted species and suppress the interference from the other species.[1] Instead of creating a standalone potentiostatic method for different potential sweeps, the arbitrary waveform method could generate almost piecewise potential function for experiments, which would facilitate greatly in the method development of electroanalytical methods.

On the other hand, building a control loop for chronoamperometric study shows benefits in a biological system. In a biological system, the growth of microbiology specimen might cause perturbation to the system. Thus, the regulation of the system could be achieved by a feedback control, eliminating any fluctuation in the system. By building a closed-loop for controlling the study, the system could measure the output and comparing it with the request, making adjustments to the parameters of the study as needed. It will alleviate the necessity of having a personal monitoring the process throughout, especially in experiments that last for weeks to complete.

The feedback control is achieved by implementing (Proportional-Integral-Derivative) PID controller in scripts within the method of the electrolysis, which is a three components controller including proportional, integral, and derivative controlling elements. In the proportional element, the adjustment to the system would be made in terms of the proportion of deviation from the ideal condition at the current iteration loop using a factor called proportion gain. In the integral element, the adjustment would be made in terms of the proportion of the integration of errors in previous loops, up to the current loop by a factor called integral gain. Lastly in the derivative element, the adjustment would be made in terms of the proportion to the derivative of the deviation at the current loop by a factor called derivative gain. Combining all these elements would give a complete correction function of PID controller.[2] In this thesis, only proportional controller was used to act as a proof-of-concept, the two other elements could be added to the feedback system in the future to provide a more comprehensive feedback correction to the system.

Implementing these features in commercial potentiostat using the compiled program provided by the manufacturer is not possible due to the fixed infrastructure of the program. Thus,

these features need to be implemented using the software development kit provided by the manufacturer. In this case, the software development kit was provided by PalmSens BV® under the MATLAB® environment for the potentiostat, EmStat3. It was chosen as a proof-of-concept, the method could be developed under different hardware, a different coding platform, but with the same ideology.

For the arbitrary waveform, we validated the method by shaping the arbitrary waveform as a triangular waveform to perform a cyclic voltammetry study in ZoBell's solution. Zobell's solution is an electrolyte first studied by ZoBell[3] containing ferrocyanide and ferricyanide in potassium chloride solution in equilibrium. It is used as a redox calibration solution for potentiostatic method because of it well-studied redox potential at different temperature. Therefore, Zobell's solution was used as a tool to compared and contrasted its result with the cyclic voltammetry experiment performed using a different potentiostat. The hypothesis is that if the arbitrary waveform method developed shows high resemblance with a close anodic and cathodic peak potential to the cyclic voltammetry data obtain from the other potentiostat, the method is working as intended. Furthermore, we demonstrated the method's ability to handle a more advanced sine function with respect to time by applying the potential waveform through an ohmic resistor and compared the result to the potential function.

For the feedback control loop, we aim to create an exemplary dynamic method which adjusts its applied potential throughout the chronoamperometry experiment by a feedback correction. In this example, we perform Oxygen Reduction Reaction (ORR) in a continuous flow nanoarray device followed by estimation of the dissolved oxygen (DO) content in the microarray device in one cycle; the estimation would be fed to the proportional gain of a PID controller to create a feedback control adjusting the potential of the next cycle. Kieninger group from the

University of Freiburg has reported Open Circuit Potential (OCP) being a method to monitor the DO content in aqueous solution.[4] Thus, the estimation of DO content in the microarray device would be accomplished by determining the OCP of the reaction mixture, serving as an instantaneous oxygen indicator in the microarray device. The sensitivity of OCP towards DO content is qualitatively determined by measuring the OCP while bubbling different percentage mixture of oxygen/nitrogen gas. It is important to establish as if the OCP is not sensitive towards DO content, resulting in the same OCP values despite varying DO content; OCP could not be used as the feedback method because it could not detect the change in the dynamic system.

Overall, both methods of the arbitrary waveform and self-adjustable electrolysis developed shed some light to provide an alternative to reinvesting potentiostat for newer or customized functions. The arbitrary waveform method is beneficial in discovering new analytical approaches to old analytical methods, increasing the method's sensitivity. Together with machine learning incorporation, it would increase the efficiency of testing different potential waveform for a higher signal to noise ratio. In the future, the direction of the development of the arbitrary waveform method should focus on improving the sensor capability of the old analytical electrochemical method to detect compounds in various sample matrices with the help of machine learning. The self-adjustable electrolysis method could be further developed or utilized for microbiology applications. It could also serve as a proof-of-concept to apply the development onto different models of the potentiostat, opening up more possibilities onto studying self-adjustable electrolysis.

2 Theory

2.1 Equivalent circuit in a potentiostat

In the electrochemical testing setup, there are three major components, working electrode, reference electrode, and a counter electrode. Randle circuit is an equivalent circuit proposed by John Edward Brough Randles to model electrochemical reaction occurring in an electrolyte considering semi-infinite diffusion of electroactive species to the electrode[5]. It has a diagram as follows,

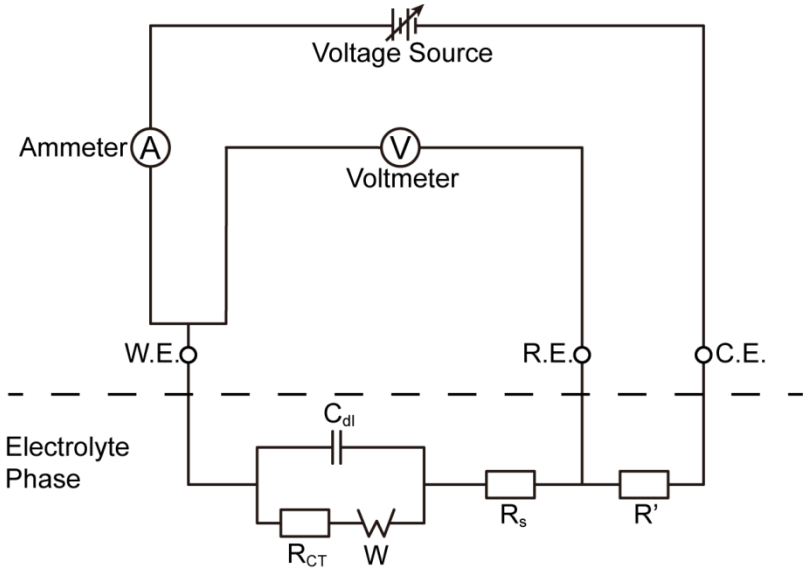

Figure 1: Equivalent circuit of a potentiostat with Randles circuit

The W.E., R.E., and C.E., are working electrode, reference electrode, and counter electrode respectively. They are the electrodes linking between the electrolyte and the potentiostat interface. C_{dl} is known as the double-layer capacitance. R_{CT} is the charge transfer resistance between the electroactive species and the electrode. W is the Warburg impedance describing the resistance to mass transfer. R_s is the series resistance between the W.E. and R.E., while R' is the series resistance between the R.E. and C.E. C_{dl} are parallel to R_{CT} and W in the Randle circuit because the total current through working eectrode surface is the sum of contributions from the

double layer charging component and the faradaic process. All the equivalent circuit components in the electrolyte interface would be introduced in detail in the later section.

2.1.1 Charge transfer resistance and Warburg impedance

R_{CT} and W contribute to the current resulting from the faradaic process. Unlike double layer charging which is only affected by C_{dl}, the faradaic process could not be represented by a single component as it is dependent on the frequency of the applied voltage. It is considered as an impedance that aroused from R_{CT} and W. R_{CT} in electrochemistry could be described as the obstacle observed when an electron is shifted from a molecule in an electrolyte onto a molecule in an electrode. It is discovered by Newbery in 1925 that there is an irreversible potential drop between the cathode and electrolyte. It is dependent on the surface of the electrode as the rougher electrode surface would result in lower values of charge transfer resistance. The charge transfer resistance is lower when the current density is higher, although the instantaneous potential drop is higher.[6] W describe the resistance to mass transfer, modeling the semi-infinite linear diffusion process on the electrode surface. It is the diffusion in one dimension, which is only bounded by a large planar electrode on one side.[7] It could be regarded as a two components system consist of a frequency-dependent resistance, $R_w = \sigma\omega^{-\frac{1}{2}}$ and a pseudocapacitance with its capacitance, $C_w = 1/\sigma\omega^{\frac{1}{2}}$. The σ in both equations is the Warburg element describes the diffusion coefficient and concentration of the redox species. From both expressions, impedance from the Warburg element decreases as frequency increases.[7]

2.1.2 Series resistance in solution

Both R_s and R' describe the solution resistance between electrodes. In a three electrodes setup, only R_s is considered to be the series resistance that needs correction but not R'. It is because electrode potential is applied between W.E. and R.E., while C.E. only acts as auxiliary

which serves to close the circuit of the electrochemistry setup. Since there is a distance between W.E. and R.E., solution resistance between them plays a role affecting the potential applied across them. The series resistance causes ohmic drop, resulting in applying lower electrode potential than intended between the electrodes, thus the electrode potential would need to be corrected to record the true potential between the W.E. and R.E. If the series resistance is low as the electrolyte is highly conductive, the ohmic drop caused by this effect is not significant because of the low series resistance when multiplied with the current density only produce a minute ohmic drop. On the other hand, if the electrode has a high surface area compared to a regular planar electrode, the huge area would produce a high current despite having a low current density. The high current would result in a significant ohmic drop when it is multiplied with the series resistance between the W.E. and R.E.

2.1.3 Double-layer effect

C_{dl} is an important characteristic of the electrical double layer present at the interface between a conductive electrode and bordering liquid electrolyte. It is contributed by two different equivalent capacitor linking in series; there are contributions from the inner and the outer Helmholtz layer of the system, and the diffuse layer capacitance derived from the Gouy–Chapman Model.[8] The inner Helmholtz Layer (IHL) refers to non-specific adsorption from the partially solvated ions or specific binding of molecules on the electrode through a chemical bond, forming a layer with a few angstrom thicknesses.[9] On the other hand, the outer Helmholtz Layer (OHL) has resulted from the pure electrostatic attraction between the IHL and ions. The diffuse layer is a layer of hydrated ions outside of the OHL, screening the net surface charge over Debye length. Both Helmholtz Layers (HL) resemble a dielectric layer between the electrode and the

diffusion layer has a characteristic capacitance property. Figure 2 is a schematic diagram showing both Helmholtz and diffuse layer on cathode surface of a potentiostat.

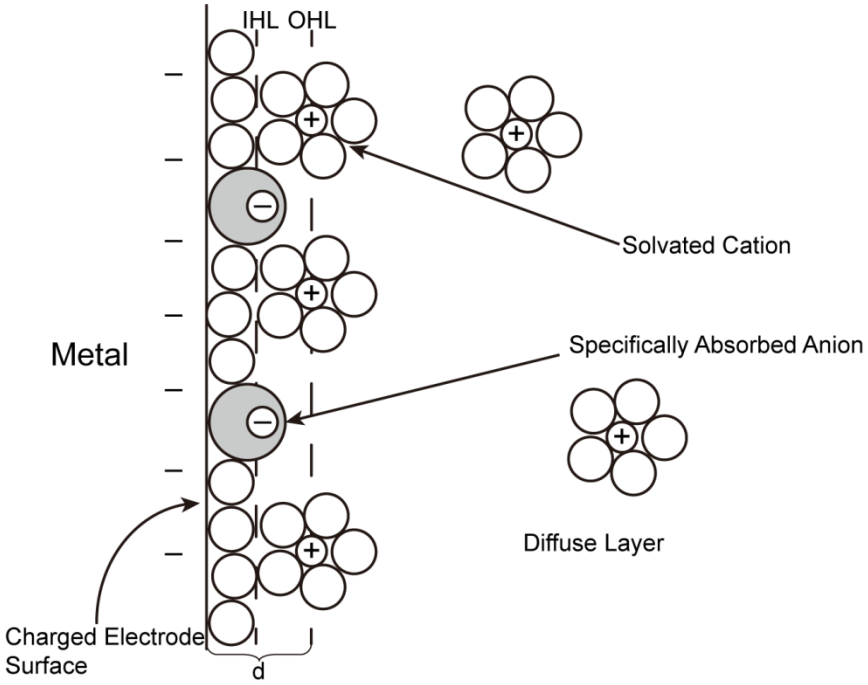

Figure 2: Double layer illustration of a cathode in an electrolytic cell taken from Figure 1.2.3 from *Electrochemical Methods: Fundamentals and Applications*[10]

According to the Gouy–Chapman–Stern model, the capacitance arises from both the Helmholtz layer is expressed as $C_H = \frac{\varepsilon_0 \varepsilon}{d}$, which resembles the capacitance from a simple capacitor over the electrode area. The capacitance arises from the diffuse layer is expressed as $C_D = \sqrt{\frac{2\varepsilon_0 \varepsilon z^2 e^2}{kT}} \cosh \frac{ze\phi}{2kT}$, which is dependent on the salt concentration and potential applied. ϕ in the equation is also known as the inner potential; it is the potential at the point of phase in the system. The relation of inner potential with the double layer is shown in Figure 3.

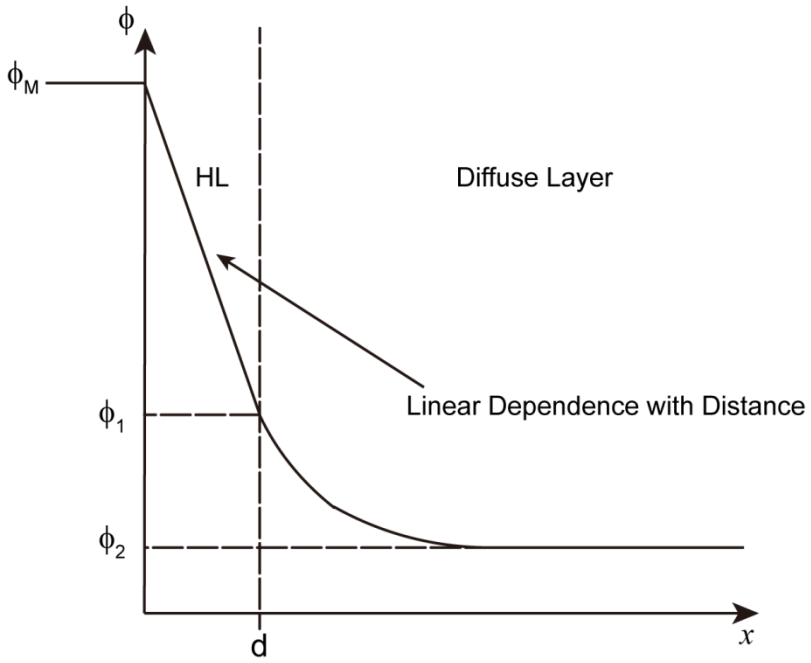

Figure 3: The inner potential change in a double layer taken from Figure 13.3.6 from *Electrochemical Methods: Fundamentals and Applications*[10]

The inner potential decreases linearly in the Helmholtz layer region as it consists of a single layer of oppositely charged ions with length, d. It acts as a single layer dielectric medium.[11] After the Helmholtz layer, the inner potential decreases non-linearly to a plateau at ϕ_2, which is the bulk inner potential in the electrolyte. The capacitance contributed by both layer are equivalently connected in series where $C_{dl}{}^{-1} = C_H{}^{-1} + C_D{}^{-1}$. At high salt concentration, the capacitance from the Helmholtz layer dominates while the capacitance from diffuse layer dominates at low salt concentration.[10]

2.1.4 Resistor-Capacitor circuit (RC circuit) in electrochemistry

As shown in the equivalent circuit diagram, C_{dl} and R_s resemble an RC circuit in solution as the voltage across C_{dl} and R_{CT} is the same due to parallel connected in a direct-current circuit. In a voltage ramp where the applied potential is linear with respect to time, the current from the

RC component is derived to plateau when current reaches υC_{dl}. The current-time behavior is graphed in Figure 4.

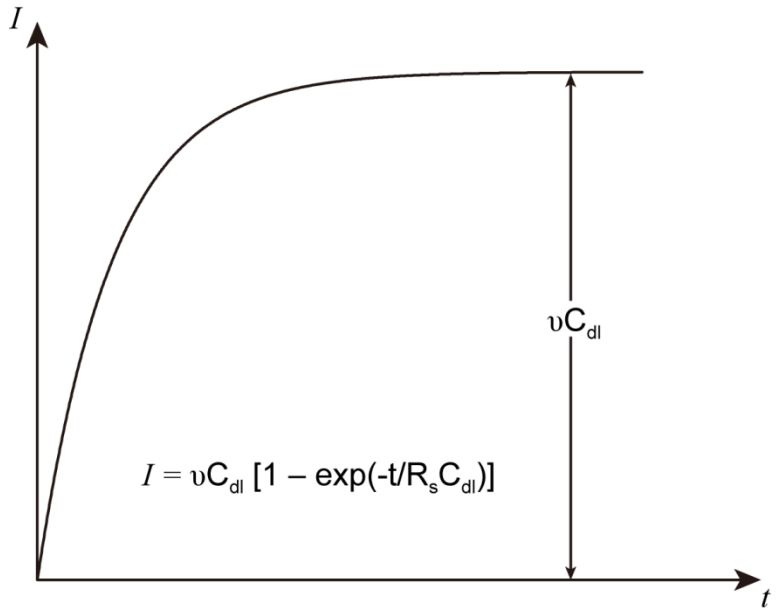

Figure 4: Current-time behavior resulting from a linear potential ramp applied to an RC circuit

At the time evolved to be much greater than RC constant, the current resulted from the double layer is constant at υC_{dl}. As the scan rate of the potential ramp increases, the current contributed from the RC component increases.

2.2 Electroanaltyical techniques

2.2.1 Cyclic Voltammetry (CV)

CV is an electrochemistry technique used to study the kinetics and mechanism of the reaction at the electrode to obtain the diffusion constants, and rate constant through the analysis of voltammogram.[12] In a conventional CV experiment, the system is scanned between a range of potentials in multiple in a triangular waveform manner as shown below in Figure 5;

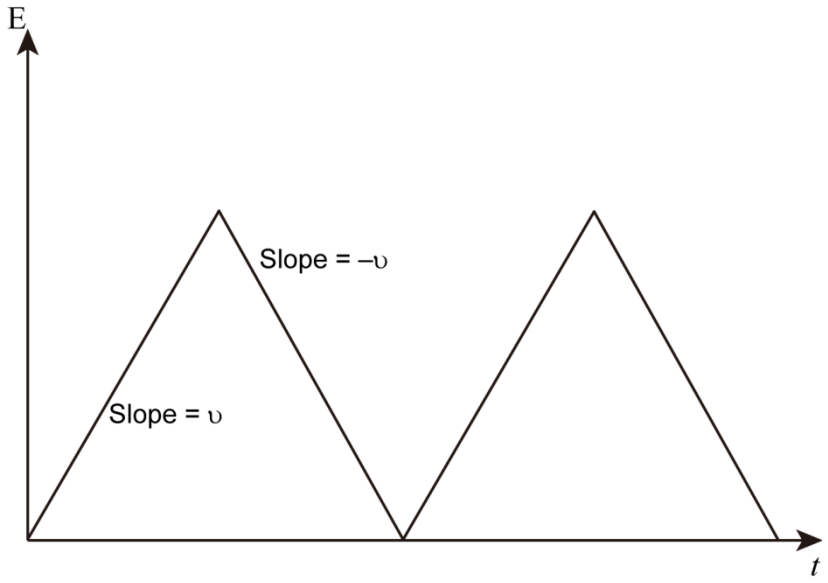

Figure 5: A schematic diagram for a triangular waveform with a scan rate of υ and –υ

Voltamogram is a plot of current against voltage applied, with general components of an anodic and cathodic peak in a reversible redox reaction. The anodic peak and the cathodic peak are separated by a peak-to-peak separation due to the diffusion of the analyte to and from the electrode, for which in a 1-electron transfer process, it is estimated to be 57 mV at 25 ℃, and given the redox reaction is reversible chemically and electrochemically. [13]

The peak of the voltammogram is described by two equations, the Butler-Volmer equation, and the Cottrell equation. The Butler-Volmer equation is derived from detailed balance and transition state theory, with the following equation;

$$j = j_0[e^{\frac{-\alpha_a F\eta}{RT}} - e^{\frac{-\alpha_c F\eta}{RT}}]$$

j is the current density at the electrode surface; j_0 is the exchange current density at the electrode surface in the absence of electrolysis; α_a and α_c are the anodic and cathodic charge transfer coefficient; F is the Faraday constant; η is the overpotential which is the voltage difference between the potential at which the experiment is conducted and the half reaction's cell potential; R is the universal gas constant; T is the temperature of the system.

Cottrell equation, on the other hand, describes the mass transport profile of the reaction over time, with the following equation;

$$j = nFc_0\sqrt{\frac{D}{\pi t}}$$

j is the current density at the electrode surface; D is the diffusion coefficient of the redox species; t is the time throughout reaction; n is the number of electrons transferred; F is the Faraday constant; c_0 is the initial concentration of the analyte species.

Combining the Butler-Volmer and Cottrell equations will yield the peak observed in a CV's voltammogram. The voltammogram at low overpotential follows the Butler-Volmer equation as the diffusion layer on the electrode surface has not developed completely. At high overpotential, the diffusion layer of the product grows to a significant size whereby the rate of the reaction is controlled by Cottrell equations. The peak current density of the voltammogram is described by the Randles-Sevcik equation with the following equation;

$$j_p = 0.4463\, nFC(\frac{nF\upsilon \mathrm{D}}{RT})^{\frac{1}{2}}$$

j_p is the peak current density; C is the concentration of analytes in the Randles-Sevcik equation; n is the number of electrons transferred; F is the Faraday constant; υ is the scan rate of cyclic voltammetry; D is the diffusion coefficient of the redox species; R is the universal gas constant; T is the temperature of the system.

Since CV involves scanning between a range of potential in with a linear potential step, the charging of the double-layer capacitor will contribute to the current in the voltammogram. Comparing the Randles-Sevcik equation and the equation governing the current contributes to the double layer, the current is proportional to the square root of scan rate and scan rate in Randles-Sevcik equation and equations governing the current contributions to the double layer

respectively. Therefore, the Randles-Sevcik equation is overwhelmed by the capacitive current due to the double layer capacitor at high scanrate. At a slow scan rate, the current density would result in a steady-state value as the current response is governed by the rate of formation of the oxidized form in the anodic region and vice versa rather than diffusion controlled at throughout the sweep, resulting in no peaks formed in the voltammogram. Thus, the common scan rate in a sweep voltammetric method normally ranges from 10 mV/s to 1000 mV/s.

2.2.2 Chronoamperometry

Chronoamperometry is an electrochemical technique where the potential applied at W.E. is constant or stepped and the current is monitored with respect to time. This function is applied usually in bulk electrolysis with constant potential, monitoring current over time. The analyte would be oxidized or reduced to another oxidation state throughout the process. As the initial analyte is consumed, the current would decrease to the baseline. The consumption of analyte is controlled by the Cottrell equation as the diffusion layer of the reacted species developed on the surface of the planar electrode; the current of the system would decrease by a factor of $t^{-\frac{1}{2}}$. The total charge of the experiment could be determined by integrating the current over time.

2.2.4 Open Circuit Potential (OCP)

OCP is used to find the resting potential of the system between W.E. and R.E. Due to the variation of experimental condition, OCP of the system is required to calibrate the system in order to perform a repeatable experiment. It is the potential at which no current is generated when it is applied between the W.E. and R.E. On the W.E. surface, the potential of the half-reaction occurring on the electrode surface is measured against the potential of the R.E.; the resultant potential is affected by the Nernst equation where the potential would be further shifted

from its reduction potential because of the concentrations of analyte, the temperature in the electrolyte as following;

$$E = E° - \frac{RT}{nF} \ln Q$$

E is the electrode potential; $E°$ is the electrode potential at standard condition; R is the universal gas constant; T is the temperature of the system; n is the number of electrons transferred; F is the Faraday constant; Q is the reaction quotient of the reaction.

Since OCP is a measure against time, the equilibrium of the system could be assumed when the OCP is stable with time. A report from NASA in 1977 shows the influence of the electrolyte environment on OCP of the system. OCP could also be applied in different fields including biosensors for different biomolecules.[14, 15]

3 Methodology

3.1 Chemicals and materials

Chemicals, unless otherwise specified, were purchased from Sigma-Aldrich and used as received. Electrodes, except nanowire array electrode, were purchased from CH Instruments, Inc. Microwire arrays were synthesized and provided by Dr. Lu from Chong's Lab according to the method he reported.[16]

3.2 Connection to the Input/Output interface of the device

The potentiostats used in the study are EmStat3 purchased from Bioanalytical Systems, Inc., and Interface 1010B™ obtained from Gamry Instruments. The software development kit for MATLAB® for EmStat3 was obtained from Bioanalytical Systems, Inc. The software development kit received contains a DLL file with compiled C++ code controlling the Input/Output interface of the potentiostat, with multiple MATLAB® function files under the same directory to access the DLL file. The DLL file with name PalmSens.Core.Matlab.dll was

loaded to the workspace of MATLAB® using NET.addAssembly() function which is only available by installing .NET Framework in Microsoft® Windows® Operating System. The device was connected to the computer via USB connection before running GetConnectedDevices() and OpenConnection() function present in the SDK package provided. The device was connected and ported to MATLAB® via variable "comm". Once the method was loaded to the workspace and configured with the correct parameters, the measurement class was utilized with Measurement() class in the existing protocol with "comm" to a variable "m". The existing protocol m.New() included in the SDK was utilized to signal the start of data acquisition. A while loop was pre-existing in the SDK was used to pause the MATLAB® code during the data acquisition. After the measurement reached completion, the measurement data stored in "m" was extracted to a structure array, converted to arrays of doubles, and exported into data files.

3.3 Arbitrary waveform validation

The arbitrary waveform method created was validated in 10 mM and 100 mM ZoBell's solution. The 10 mM ZoBell's solution is composed of 10 mM $K_3Fe(CN)_6$, 10 mM $K_4Fe(CN)_6$, and 0.1 M KCl prepared in a 50 mL centrifuge tube. The 100 mM ZoBell's solution is composed of 100 mM $K_3Fe(CN)_6$, 100 mM $K_4Fe(CN)_6$, and 0.1 M KCl prepared in a 50 mL centrifuge tube. The redox reaction in ZoBell's solution is shown below with redox potential of 0.1 M and 0.01 M ZoBell's solution reporting to be 0.4586 V and 0.4154 V *vs.* SHE respectively.[17]

$$Fe(CN)_6^{3-} + e^- \rightleftharpoons Fe(CN)_6^{4-}$$

Once the ZoBell's solution was prepared, they were used immediately or stored in the dark to minimize exposure to the light so the redox potential of the solutions was not altered significantly. The ZoBell's solutions were used as the electrolyte for arbitrary waveform

validation. The validation was conducted using a 3.2 mm-diameter glassy carbon electrode as W.E., Ag/AgCl reference electrode as R.E., and platinum wire as C.E.

3.4 Self-adjustable electrolysis validation

The self-adjustable electrolysis method was created and validated in Phosphate-buffered saline (PBS) medium, which compose of 0.137 M NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 1.8 mM KH_2PO_4 with pH adjusted to 7.4. Before electrolysis, the medium was saturated with air by bubbling air in the medium for 30 minutes. The electrolysis method was validated using a microwire array electrode with a syringe pump to feed medium through the chamber at a constant rate. Microwire array is used in this validation as the ORR on the microarray electrode surface creates a local oxygen gradient with a high surface area electrode.[16] In the setup, the microwire array was used as the W.E., a piece of silver paint on the ITO glass which the microwire array is resting was used as the R.E., and a platinum wire was used as the C.E. in this study. The silver paint reference electrode was a pseudo-reference electrode where its redox potential is around 0.32 V *vs.* SHE[16]. A syringe pump (New Era Pump Systems, Inc.) was used to feed the liquid medium into the chamber of the microwire array at a flow rate of 2 mL/min.

3.5 Open Circuit Potential (OCP) sensitivity test

An experiment was performed to examine the sensitivity of OCP towards DO content in PBS buffer. PBS buffer prepared previously was added to a glass cell in a three-electrode setup consisting Ag/AgCl reference electrode as R.E., platinum wires as W.E. and C.E. respectively. The set up was then sealed air-tight with parafilm. Plastic tubings of gas inlet and outlet were inserted into the setup. The setup was connected to EmStat3; An OCP method was created in MATLAB® for a total runtime of 1 s with an interval time of 10 ms. The PBS buffer in the cell

was bubbled in the various composition of oxygen/nitrogen mixture from 5 – 20 % partial pressure of oxygen for 10 minutes. OCP was run after stopping the gas flow.

4 Results and Discussion

4.1 Design Scheme

As the method of arbitrary waveform does not exist in the methodology library of the potentiostat, the method was developed with a multistep chronoamperometry method. The total time of the run was segmented and generated a linear spaced vector with a small time interval. The timestamp of the vector was substituted to the equation of the potential waveform to generate a vector of potentials to be applied with the time interval. These potentials were written into a method of multistep chronoamperometry by creating a loop adding the potential level sequentially into the method. A simplified model is demonstrated below;

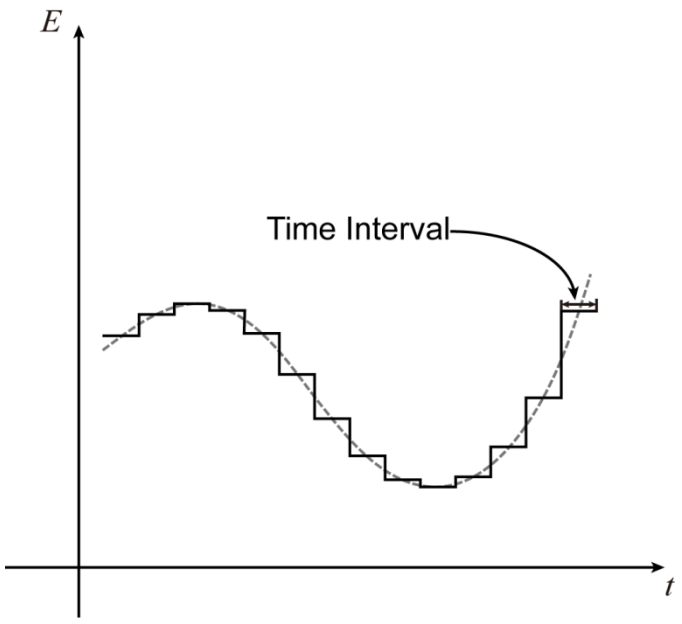

Figure 6: Arbitrary waveform implementation

In the above figure, the grey dotted line is the potential waveform function whereas the black line represents the potential steps applied throughout the experiment. The time interval is lower significantly to increase the temporal resolution in order to show a high resemblance to the

potential waveform. This technique is only achievable with MATLAB® because the built-in software developed by the manufacturer, PSTrace, is only capable of creating a maximum number of 255 levels; it is not sufficient levels to create an arbitrary waveform method using PSTrace, given that a 5 seconds experiment with 1-millisecond interval would need 5000 levels.

The idea of self-adjustable electrolysis is originated from controlling the parameters of electrolysis through feedback control of the readout signal. The readout signal could be obtained in various forms from chromatographic measurements like gas chromatography of the headspace of the electrolyte to spectroscopic measurements like ultraviolet-visible spectroscopy to the electrolyte. However, those implementations would require coupling other instruments with the potentiostats. This simple readout signal constructed based on the relationship between OCP and DO content in the electrolyte only serves as an example of how the self-adjustable electrolysis could be implemented. As ORR is performed as a chronoamperometry technique in solution, the dissolved oxygen is reacted via either a 2 or 4 electrons pathway to form hydrogen peroxide and water respectively. OCP will be performed as a DO indicator to monitor the DO content in solution. Using the DO content detected, a mathematical model will be developed to control the parameters of the next chronoamperometry. The microwire array developed in our lab was used as a model to develop and study the method. Since OCP could only determine the DO content at the surface of the electrode, the total DO in the bulk solution does not change significantly over a short period. There is an advantage to use microwire array because the morphology of the microstructure of the microwire array hindered the mass transport of the DO content in the electrolyte. There is less effective mass transport, achieving a controllable DO concentration within the microwire array. If the experiment were conducted in bulk electrode and solution, the oxygen concentration near the electrode surface would be homogenized rapidly. A further design

of the experimental setup would be required to facilitate the acquisition of the feedback signal. For example, the experimental setup could be placed in an ultraviolet-visible spectrophotometer, where it is connected to the computer simultaneously with the potentiostat. The data generated from the spectrophotometer could act as a feedback signal for controlling the parameters of the electrolysis, achieving the goal of "self-adjustable".

Unlike arbitrary waveform which was developed and existed as a standalone method, the method of self-adjustable electrolysis was written as a script, acquiring and store data as the loop run. There are two parts of the loop; the first part is the electrolysis phase, while the latter is the feedback phase. Methods of chronoamperometry with a starting potential and OCP were defined and stored in the workspace of the MATLAB® console together with the targeted OCP which could be obtained through a calibration curve measuring the OCP at different DO concentration. In the electrolysis phase, the chronoamperometry method was run at the starting potential for a period of time. As the electrolysis phase ends, OCP was run subsequently for a much shorter period to determine the DO content in the solution in the feedback phase. Once the OCP data is acquired, it is compared to the targeted OCP to determine the error between the targeted and measured OCP. P Gain of control theory was used as feedback control for the voltage. It is one part of the three-term controller, PID controller, which is implemented in the electrolysis method to control the voltage applied for the next loop. The loop continued to the end of the experiment. Optimum voltage would be maintained as the system reaches steady state close to the ideal voltage. A simplified scheme is shown in Figure 7.

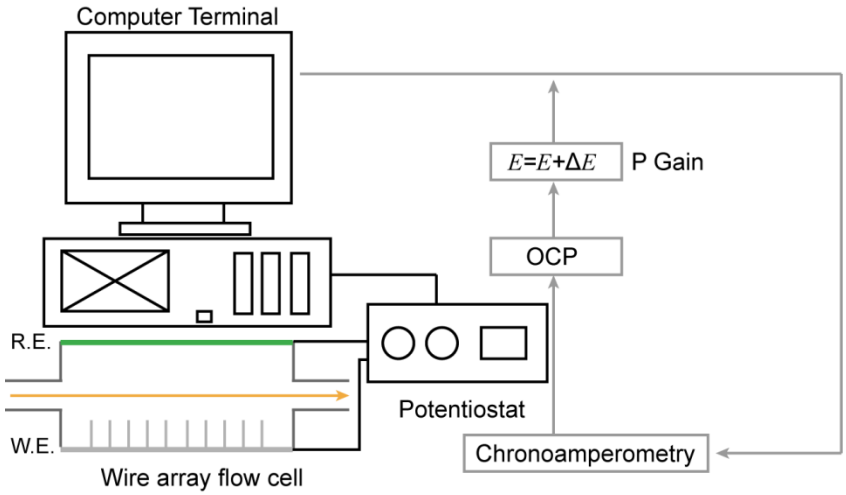

Figure 7: Self-adjustable electrolysis scheme

4.2 Arbitrary Waveform

The method created initially was tested with an ohmic resistor of 10,000 Ω using a sine potential function and shown in Figure 8 below.

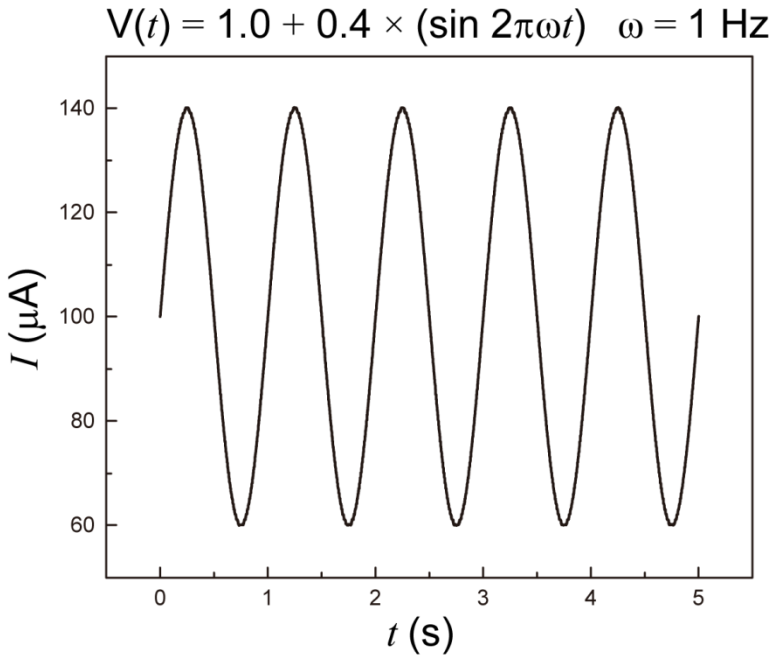

Figure 8: Current response from a sine wave potential from t = 0 s to t = 5 s

The initial experiment did not finish within the time specified but persisted through a longer time length. It is suspected that the method lags between each interval, causing

asynchronism between the current-time graph and the real time. The asynchronism might arise from the lag time in communication between the potentiostat and the computer terminal. Assuming the lag interval between each step is the same, quantification of the lag interval was made possible by timing the length of data acquisition in MATLAB® using the timer function. The average error present in 2 distinct intervals setting, 1 millisecond and 100 milliseconds, were tested and tabulated in Table 1. The interval of 100 milliseconds was tested with a runtime parameter of 60 and 300 seconds to create 600 and 3,000 levels respectively; the interval of 1 millisecond was tested with 5 and 10 seconds to create 5,000 and 10,000 levels respectively.

Table 1: Determination of synchronization error in the method

	100 milliseconds interval		1 milliseconds interval	
	60 seconds runtime	300 seconds runtime	5 seconds runtime	10 seconds runtime
Actual Run Time Measured (s)	95.05 ± 0.11	464.84 ± 0.09	159.98 ± 0.10	319.81 ± 0.08
Error Calculated (ms)	58.42 ± 0.07	54.94 ± 0.01	31.00 ± 0.02	30.98 ± 0.01

Table 1 showed the error present varied in different interval settings. Thus, the arbitrary waveform method needs to be calibrated for the synchronization error before use.

After incorporating the error into the method, the finest temporal resolution achieved for this method is around 32 ms. Considering the hardware limitation of the maximum step potential of 250 mV given in the manual of the potentiostat[18], the maximum rate of potential change possible was calculated to be around 8 V/s. Comparing it to the scan rate window in LSV/CV, the maximum rate of potential change in the arbitrary waveform method is fully applicable in LSV/CV.

The arbitrary waveform method was demonstrated in Figure 8 by applying a more complex sine potential waveform to the ohmic resistor to show that the error calculated does not deviate throughout the experiment, causing an error in synchronization.

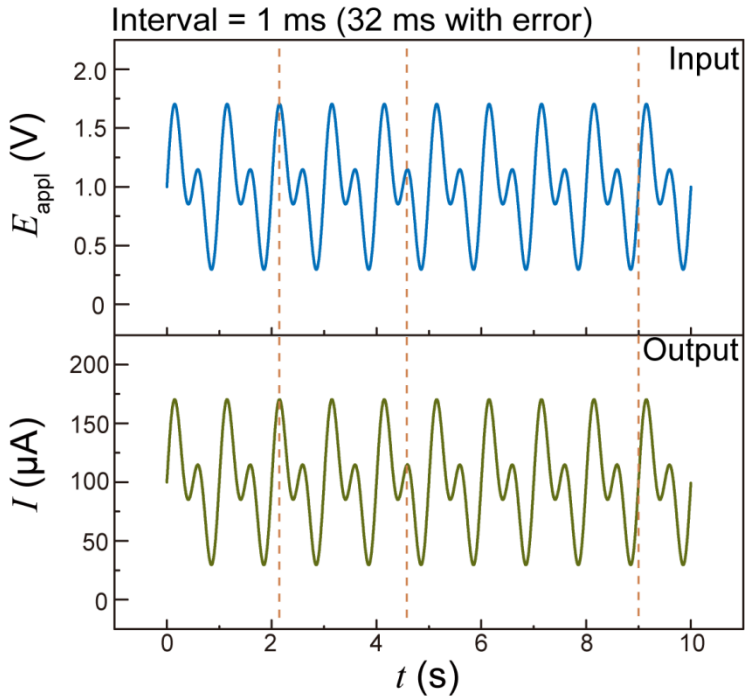

Figure 9: Complex sine waveform input and current response

The potential waveform applied to a 10,000 Ω ohmic resistor in Figure 7 is V(t) = 1.0 + 0.4 × (sin(2πωt) + sin(4πωt)). The orange dotted line showed both input and output graphs synchronized perfectly. The maximum and minimum instantaneous gradient of this potential waveform was determined mathematically to be 7.5 and – 5.2 V/s respectively; these rates are less than the theoretical maximum potential rate calculated earlier. Thus, there exists a possibility where the method will fail if the gradient of the waveform is greater than that of the theoretical maxima, and should be investigated further to optimize the method.

Since LSV could be expressed in a triangular waveform, arbitrary waveform could be used to model the triangular waveform. However, in CV, the voltage function is a continuous function with a scan rate of υ and – υ; whereas, in the arbitrary waveform, the voltage applied

resembled a digital staircase where the voltage function as a piecewise function of potential constant mimicking a triangular waveform. Although digital staircase is frequently used in the electrochemical instruments industry as most of the potentiostats available in the market are digital instruments with digital signal generators[19], there exist uncertainties of the arbitrary waveform method on the width of the staircase due to the error in synchronization, causing uncertainty in sampling time. The voltage per step is only applied for a short duration while the remaining time of the step is "potentialess". The feasibility of the method as a CV should be investigated as the difference between the digital staircase of a regular CV and arbitrary waveform would affect the response current. Thus, a one cycle CV method was created using the arbitrary waveform method with the response current comparing with that of a traditional CV produced in Gamry. Both CV methods were conducted in 10 and 100 mM of ZoBell's solution, scanning a cycle between 1.097 and – 0.403 V *vs.* SHE, and the resultant voltammograms were overlapped to observe if both methods produce a similar result. ZoBell's solution is used because of its stability as a redox calibrator with a standard potential reported to be 0.430 V vs. SHE for a 3.33 mM ZoBell's solution.[3] Figures 10 and 11 showed the voltammogram at different scan rates of 10 mM and 100 mM ZoBell's solution respectively.

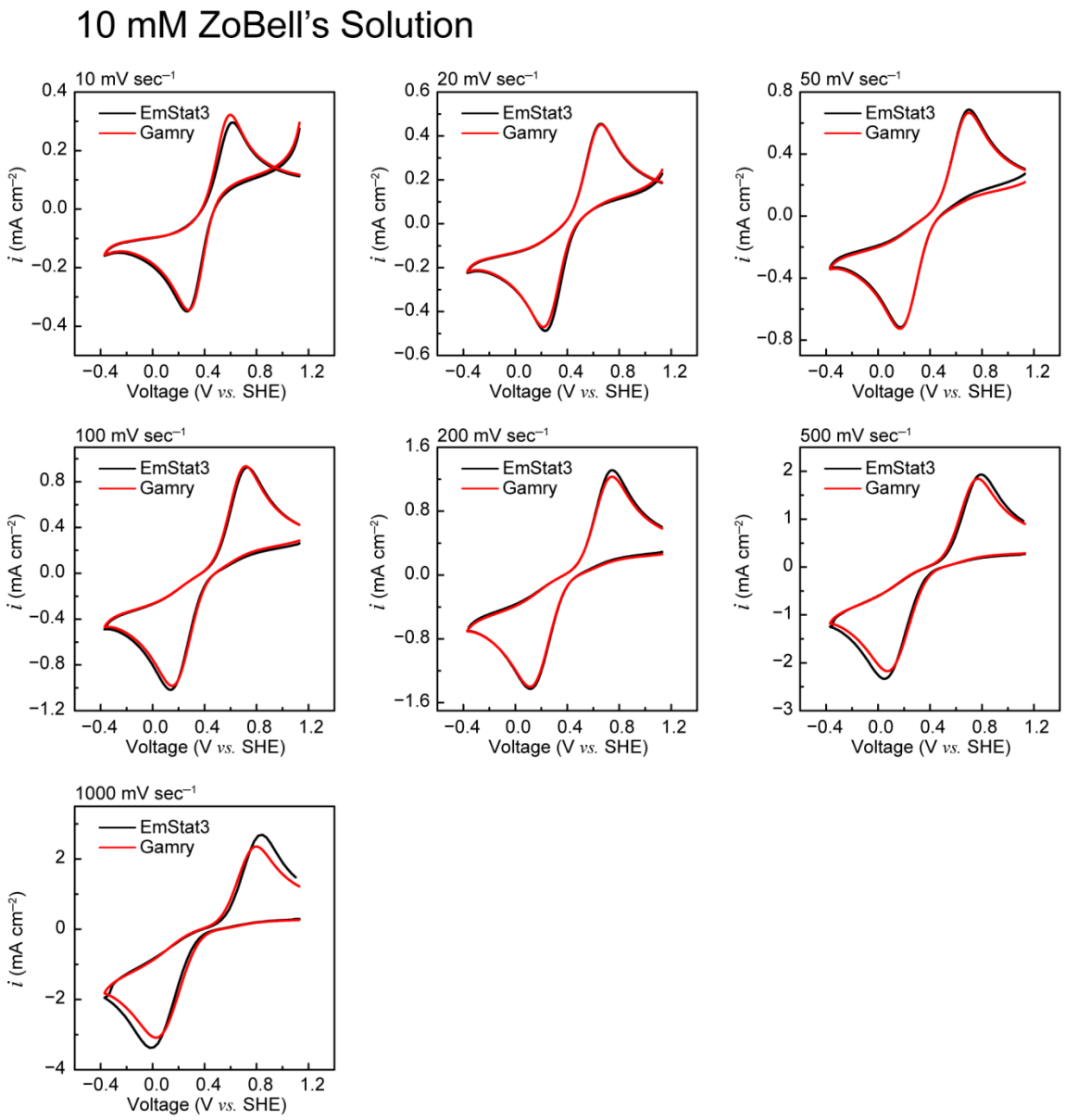

Figure 10: Voltamogram of 10 mM ZoBell's solution at scan rate ranging from 10 to 1000 mV/s

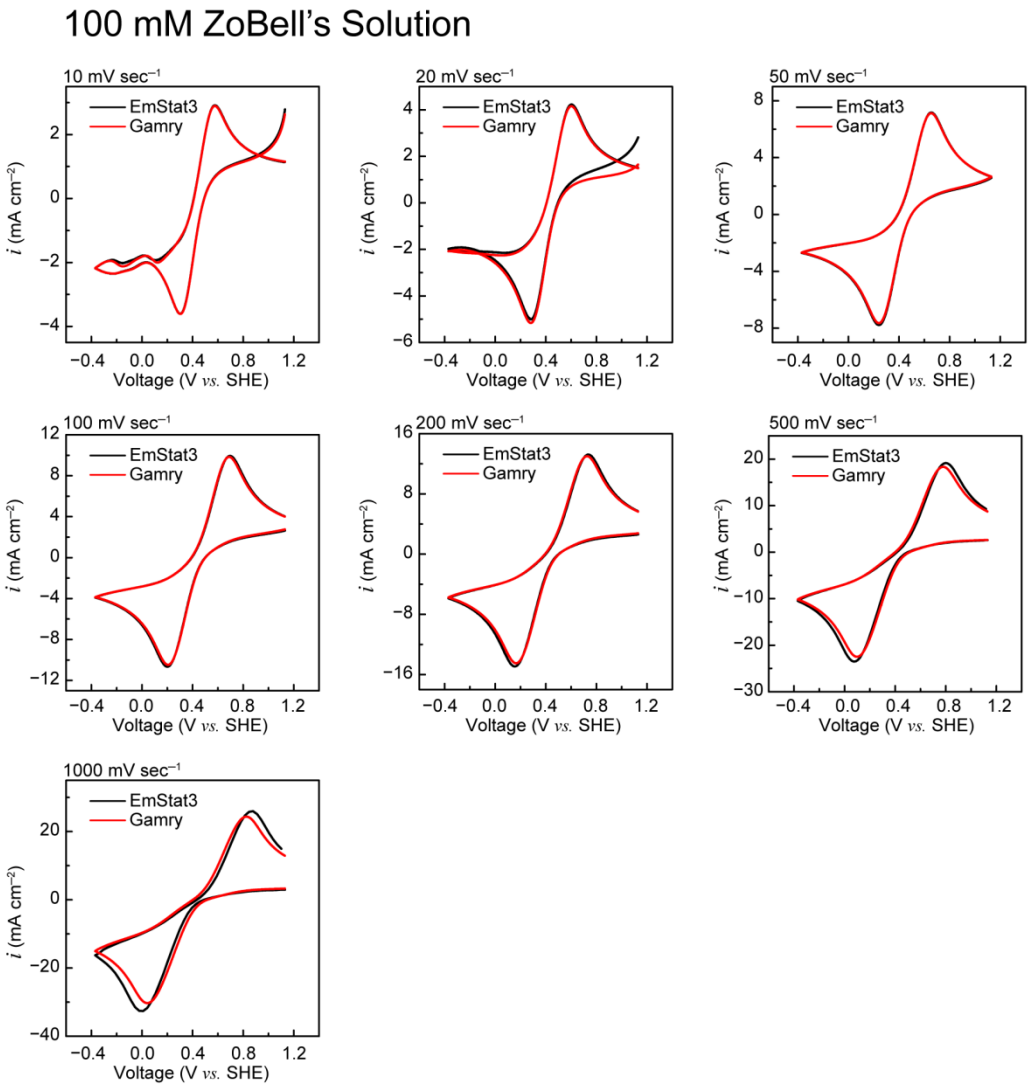

Figure 11: Voltamogram of 100 mM ZoBell's solution at scan rate ranging from 10 to 1000 mV/s

The red lines in both figures represent the voltammograms generated using Gamry Interface 1010B, while the black lines represent the voltammograms generated using arbitrary waveform constructed in MATLAB® using PalmSens EmStat3. The anodic and the cathodic peak potential are tabulated in Table 2 with the absolute error between the arbitrary waveform and Gamry on the cathodic and anodic peaks tabulated in Table 3.

Table 2: The anodic and the cathodic peak potential from both potentiostats for different scan rate and concentration of Zobell's solution

10 mM ZoBell's solution				
	Cathodic Peak (V vs. SHE)		Anodic Peak (V vs. SHE)	
	Gamry	Emstats3	Gamry	Emstats3
10 mV/s	0.2449	0.2285	0.5650	0.5839
20 mV/s	0.1839	0.1965	0.6299	0.6306
50 mV/s	0.1360	0.1418	0.6670	0.6674
100 mV/s	0.1189	0.1082	0.6860	0.6978
200 mV/s	0.0810	0.0794	0.7110	0.7106
500 mV/s	0.0440	0.0090	0.7400	0.7650
1000 mV/s	-0.004	-0.0550	0.7689	0.8130
100 mM ZoBell's solution				
10 mV/s	0.2709	0.2698	0.5430	0.5458
20 mV/s	0.2500	0.2477	0.5700	0.5730
50 mV/s	0.2079	0.2074	0.6219	0.6210
100 mV/s	0.1740	0.1658	0.6570	0.6626
200 mV/s	0.1350	0.1242	0.6980	0.7042
500 mV/s	0.0680	0.0410	0.7500	0.7650
1000 mV/s	0.0130	-0.0230	0.7959	0.8450

Table 3: Absolute error between both potentiostats on the cathodic and anodic peaks for different scan rate and concentration of Zobell's solution

10 mM ZoBell's solution		
	Absolute difference in Cathodic Peak (V vs. SHE)	Absolute difference in Anodic Peak (V vs. SHE)
10 mV/s	0.0164	0.0189
20 mV/s	0.0126	0.0007
50 mV/s	0.0058	0.0004
100 mV/s	0.0107	0.0118
200 mV/s	0.0016	0.0004
500 mV/s	0.0350	0.0250
1000 mV/s	0.0510	0.0441
100 mM ZoBell's solution		
10 mV/s	0.0011	0.0028
20 mV/s	0.0023	0.0030
50 mV/s	0.0005	0.0009
100 mV/s	0.0082	0.0056
200 mV/s	0.0108	0.0062
500 mV/s	0.0270	0.0150
1000 mV/s	0.0360	0.0491

In 10 mM ZoBell's solution, the absolute difference between Gamry and Emstats3 in the cathodic and anodic peak at scan rate between 10 to 200 mV/s were below 20 mV, whereas in 100 mM ZoBell's solution, the absolute difference between 10 to 100 mV/s were below 10 mV. The highest degree of overlap in voltage occurred in 100 mM ZoBell's solution at 50 mV/s with the Emstats3 peak voltage only differs from the Gamry's by 0.5 and 0.9 mV in cathodic and anodic peak respectively. At a higher concentration of ZoBell's solution, the arbitrary waveform method was as responsive as the CV in Gamry.

At a higher scan rate, the peak voltage did not overlap as well as the lower scan rate as the difference increased significantly because of the way the arbitrary waveform method was structured. As we have discussed earlier, the finest temporal resolution achieved for the arbitrary method is 32 ms; at a scan rate of 1000 mV/s, the voltage interval between each stair is 32 mV which scanning a cycle between 1.097 and – 0.403 V *vs.* SHE only produces 46 voltage steps in the forward scan, 92 voltage steps in total, equivalent to 92 data points collected throughout the experiment. It is a rough staircase voltammetry compared to the CV created in Gamry which consists of 3001 points. Unlike Gamry, the resolution of CV in the arbitrary waveform is greatly limited by the fixed temporal resolution of the method; thus, this method did not provide ideal modeling of the triangular waveform as the potential steps are significantly rougher at higher scan rate. From both figures at a high scan rate, the onset voltage for the arbitrary waveform method lag the onset voltage of CV because of the larger temporal resolution of the arbitrary waveform method. A smaller temporal resolution would provide a more sensitive voltage control throughout the experiment.

Arbitrary waveform ideally could be used to increase the sensitivity of the electroanalytical method. In past research, the Compton group had shown cosine based potential

waveform could be used in place of a triangular waveform in a CV to prevent non-continuous capacitive perturbation to the system as it prevents the discontinuity of the scan rate during the switch of forward and backward scan[20]. They also showed a semi-circular potential waveform with a local infinite scan rate amplify the peak current of the redox species while suppressing the interference of the other species[1]. Comparing to their potentiostat which was built bottom up[21], this method created an easier alternative to reproduce the potential waveform. Arbitrary waveform method could generally be applied to sweep voltammetry where a more sensitive waveform is developed for electrochemical analysis. The increase in sensitivity could also be applied in the field of sensors. There was a report using CV as a gas sensor for methane and other volatile organic compounds[22]. The triangular waveform in CV could further optimized for a more sensitive method with a higher signal-to-noise ratio. The method could be developed further with machine learning, which various potential waveforms are tested and used as training data to screen for a more sensitive method.

4.3 Self Adjustable Electrolysis

The method of electrolysis of ORR monitored by DO content in the electrolyte was generated using the design schemed discussed in the earlier section. Error in synchronization would not occur as the potentiostat is not scanning rapidly over a potential range. To ensure the OCP is sensitive to DO content, the OCP sensitivity test mentioned in the method section was performed by bubbling various composition of oxygen/nitrogen gas mixture into a bulk electrochemical cell measuring its OCP in the electrolyte. The obtained data were plotted against DO content in the figure below where it is calculated using salinity of the PBS buffer, Henry's law constant, and the partial pressure of gaseous oxygen.

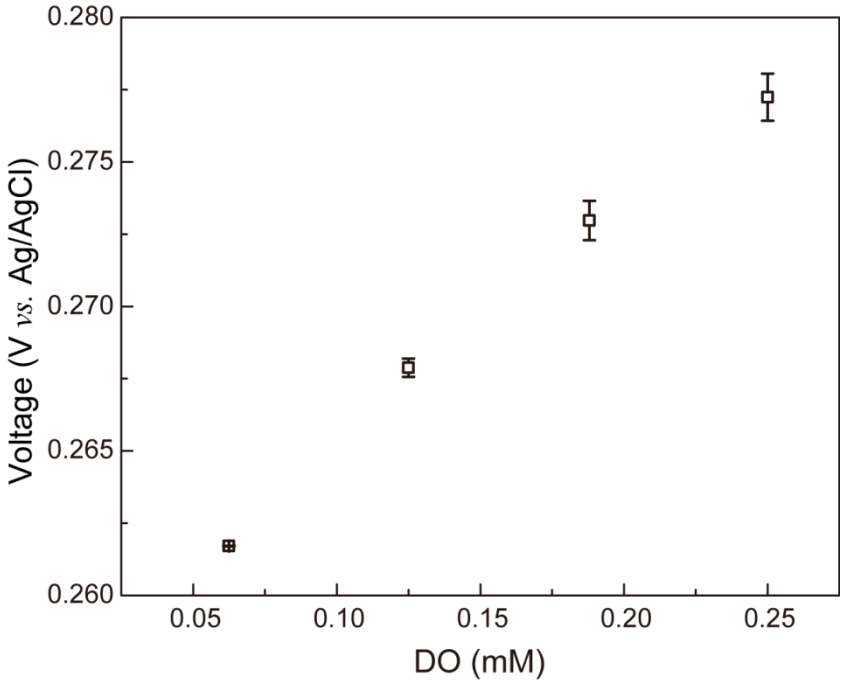

Figure 12: OCP sensitivity test is done in replicates of three

From Figure 12, OCP of the cell is responsive over a small range of voltage for DO. One fold increase of DO from 0.125 to 0.25 mM DO results in a 9 mV increase. This method will provide a rough estimate of DO in solution. Theoretically, the trend of the plot should follow the Nernst equation, as the concentration of DO increases logarithmically, the potential of the ORR reaction should increase. Using OCP as a way to estimate the cell potential of ORR is only valid if the majority of the reaction present is assumed to be ORR, and there is no significant side reaction taking place in the cell. Based on our result, OCP was proven to be responsive towards the change in DO and could serve as an indicator for DO content in the electrochemical cell.

After determining OCP could serve as an indicator for DO content in the electrochemical cell, the development of the method was continued using a microwire array flow cell instead of a bulk glass cell to test the feasibility of the method. The microwire array was connected to EmStats with air saturated PBS medium feeding through the chamber of the cell. Before implementing P Gain into the method, the method was performed to check the consistency of the

method under constant voltage. The starting voltage, – 0.09 V *vs.* Ag/AgCl, was determined using the stimulation data on the microwire array published in Lu's paper[16], as it could provide an oxygen gradient on the surface of the microwire array. Both chronoamperometry and OCP were created in MATLAB® workspace with an individual runtime of 59 s with 1 s interval and 1 s with 10 ms interval respectively. The time taken for one complete loop would take 1 minute. The loop was repeated 10 times to check the consistency of the method developed without the implementation of a feedback control. The OCP obtained in one of the loops were plotted below in Figure 13 and examined.

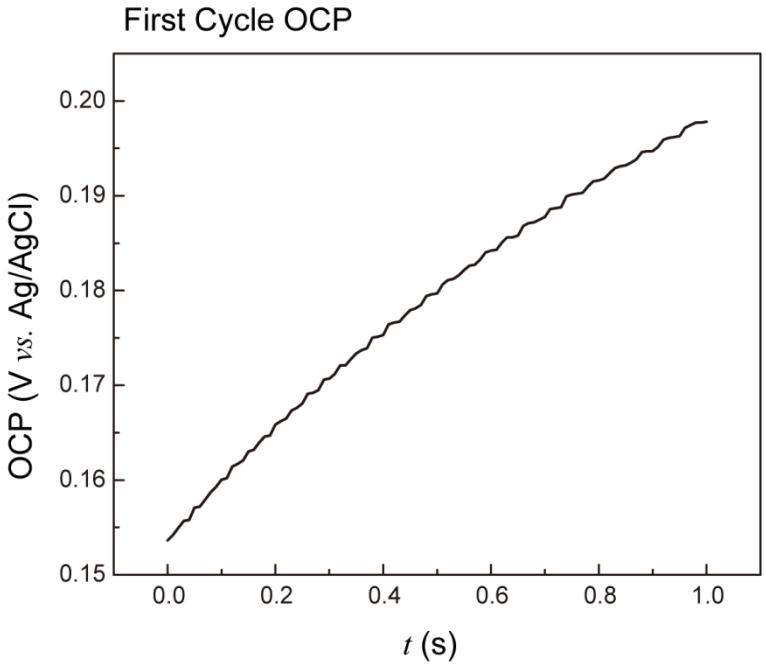

Figure 13: OCP measurement of 1st loop

Throughout the measurement, OCP increased rapidly over 1 s. The increase in OCP is because of the diffusion of oxygen in the chamber towards the working electrode surface, increasing the OCP measured with respect to time. In the method, the electroanalytical methods were implemented in sequence run where there is a time delay between the end of a method and the start of the other method. This delay is inevitable where the first data point in the OCP

method was acquired after a brief delay when the method is switched. It is assumed the OCP of the microwire array increased immediately after the chronoamperometry ends as the potential applied stopped; thus, the OCP had already shifted from its original value when the OCP method started. Since the initial value of OCP was not obtained, the first value of the OCP method would be the closest estimate of the DO content in the cell instead of the average OCP in the individual experiment. The initial value of OCP was used as the feedback OCP of the method. After obtaining the feedback OCP from all 10 loops, they were plotted against the number of loops in the figure below; ideally, the OCP would remain constant throughout all loops as the system reached steady-state rapidly.

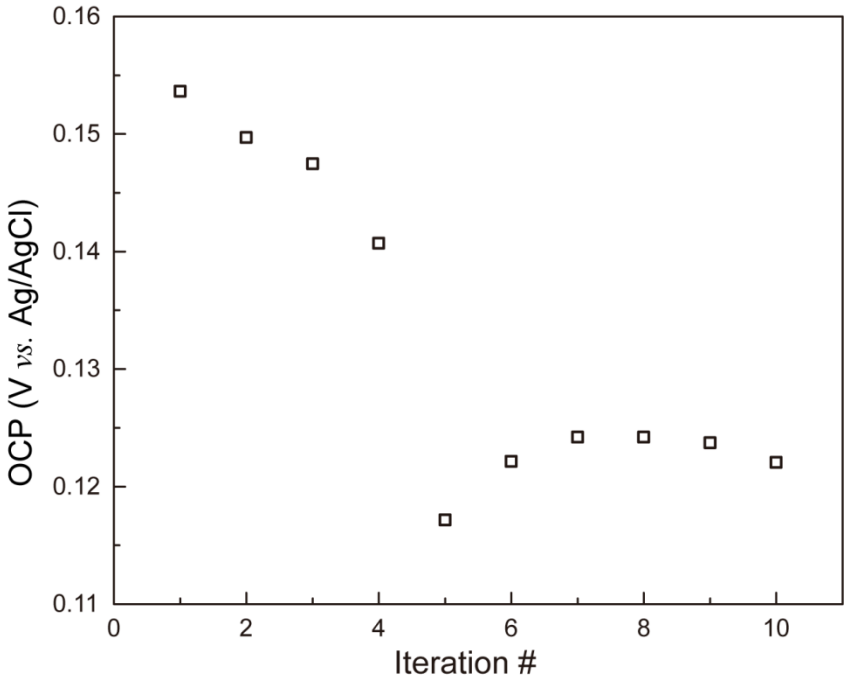

Figure 14: OCP measurement for 10 loops using the initial value of the OCP for each iteration without the implementation of feedback control

In Figure 14, the OCP measurement of the first four loops had a decreasing trend and shown not consistent. This indicated the system had not yet stabilized; the concentration of DO

on the surface of the microwire array electrode is changing with time. From the fifth iteration, the OCP of the system stabilized at 0.122 ± 0.003 V *vs.* Ag/AgCl. Thus, the parameter of chronoamperometry runtime was amended from 59 s to 599 s with 1 s interval to ensure the system is stabilized. The resultant loop would have a total runtime of 600 s with 1 s OCP probing.

P Gain was introduced into the loop by first defining a gain constant, K_p, and the target OCP. In the feedback phase, the first data point of OCP in that loop was extracted to a variable in MATLAB® workspace. The error was calculated by subtracting the target OCP from the first OCP. The potential for the next loop was calculated by subtracting the current potential with the product of error and K_p. As the error reduces after every loop, the potential for the next loop would deviate lesser from the ideal potential until the error between the potential recorded and the ideal potential is less than the threshold of error. To express the mechanism, an infinite loop was implemented with the conditions stated in Table 4.

Table 4: Parameters of the self-adjusting electrolysis

Parameters of Method	
Starting Potential (V *vs*. Ag/AgCl)	0.41
Target OCP (V *vs*. Ag/AgCl)	0.15
K_p	1.5
Break Loop Condition	|Error| < 0.005

The infinite loop was run and stopped at the fifth iteration with the potential applied and the first OCP values plotted against the instance of iteration in Figure 15.

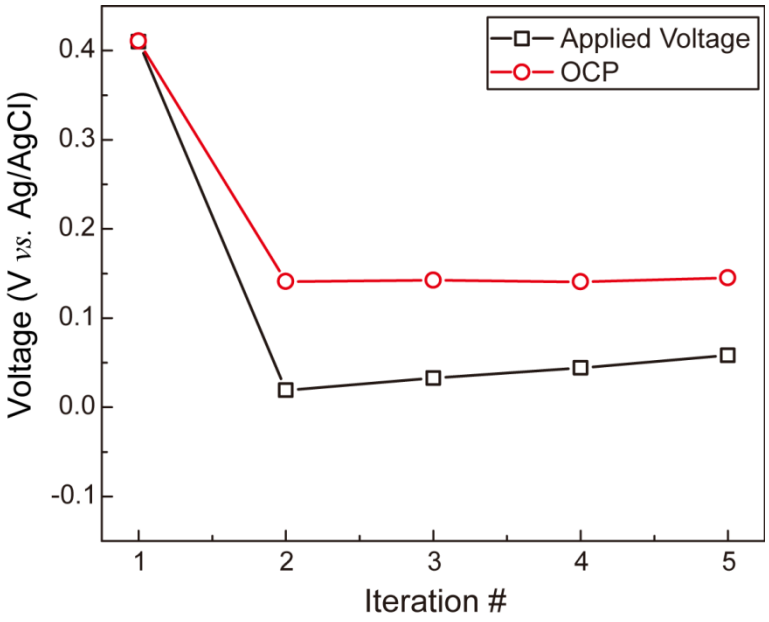

Figure 15: Applied voltage and initial value of OCP at different iteration with the implementation of feedback control using the parameters described in Table 4

The loop halted successfully as the error of the OCP deviated less than 0.005 V from the target OCP. It showed the capability of the method self-adjusting its potential to the ideal potential for bulk electrolysis. Only proportional gain was used in this method development; however, there are two other terms in the PID controller, integral and derivative terms could be implemented to the method[23], possibly gaining more precise control over the potential adjustment.

The developed method could be applied in the biological living system in which the microwire array environment might changes gradually with time. The change in environment would cause shifting in the potential of species according to the Nernst equation. With the self-adjusting element, the experiment could be performed remotely without mandatory personnel controlling parameters constantly. It could be used if the biological system requires stringent conditions to maintain. For example, if the bacteria grow optimally at 5 mM DO, this method would provide such conditions after equilibrium. The self-adjustment data could in the future

used as training data for machine learning to determine the optimized parameters to achieve a more cost-efficient method with lesser side reaction.

However, there exist limitations to the developed method. The OCP indicator developed failed to detect the exact concentration of DO present in the solution right after the electrolysis phase. Calibration of the concentration of DO could be performed by coupling the method with confocal microscopy studies[16]. Moreover, OCP is a rough estimate of DO content assuming there is no other side reaction in the solution. If there are other redox couples in the solution which gave an intense signal, the method developed is not applicable to estimate DO content. Nonetheless, the feedback phase could be replaced with other probing techniques to probe oxygen in the system. For example, Gabriel group has built a DO microsensor using gold microelectrode.[24] This microsensor could be inserted into the system and perform a more accurate DO measurement of the system using a multiplexer potentiostat system.

5 Conclusion

Both methods of the arbitrary waveform and self-adjustable electrolysis were successfully developed for EmStats3 in MATLAB® using the SDK provided by the manufacturer. The drawbacks of the arbitrary waveform method include the hardware limitation of the maximum step potential and the fixed temporal resolution which leads to a coarse potential staircase in a high scan rate setting. In addition, the difference in the way the current is sampled might produce in strikingly different results especially the capacitance effect. Further investigation should be done to analyze the effect of the potential staircase we developed to the capacitance effect. The self-adjustable electrolysis method developed would allow users to utilize the closed-loop designed to control chronoamperometric study, where the system could measure the output during the feedback phase and comparing it with the target, adjusting

parameters as needed. It provided another approach for researchers to fine-tune their experiment system automatically without physical interference. The limitation of this method arises from the uncertainty of OCP detection as DO concentration increase near the electrode surface as diffusion to the depleted region took place when the potential was not kept. The delay in the detection of OCP might cause the OCP to deviate from its value when the measurement took place. Furthermore, it is assumed the major species in the electrolyte is the redox couple of ORR; if the electrolyte environment was changed vigorously, this assumption might no longer hold. Nonetheless, OCP measurement gives a rough estimate of DO content in the electrolyte which could be used as an indicator for auto-adjustment. The feedback phase could also be switched with other electroanalytical techniques to create methods with other feedback mechanisms.

Overall, both methods developed shed some light to provide an alternative in a reinvestment of potentiostat for newer or customized functions, elongating the service life of existing potentiostat. The arbitrary waveform method could be beneficial in discovering new analytical approaches to old analytical methods, increasing the method's sensitivity, or simplifying the analytical method. Especially in arbitrary waveform method, machine learning could be incorporated with arbitrary waveform, greatly increases the efficiency of testing different potential waveform for specific application for a higher signal to noise ratio. A similar approach could be taken for different potentiostat to develop new methods respectively. The arbitrary waveform method developed theoretically would allow users to test the current response of any potential waveform. In the future, apart from solving the drawbacks incurred with the development of the method, the direction of the development of arbitrary waveform method should focus on improving the sensor capability of the old analytical electrochemical method to detect compounds in various sample matrices with the help of machine learning. The

self-adjustable electrolysis method could be further developed to incorporate a more accurate readout measurement in the feedback phase to increase the accuracy of the feedback control, generating a calibration curve for the determination of DO in the microarray system. The method could also further developed to enable other modifications of parameters like the temperature of the experiment using a thermostat apart from modifying the electrode potential applied. The method could also be applied directly towards the research of microbiology by loading bacteria strain to the microwire array and perform microbiological studies on the bacteria in various DO conditions.

6 Reference

(1) Uchida, Y.; Kätelhön, E.; Compton, R. G., Linear sweep voltammetry with non-triangular waveforms: New opportunities in electroanalysis. *J. Electroanal. Chem.* **2018,** *818*, 140-148.

(2) Unbehauen, H. D., *CONTROL SYSTEMS, ROBOTICS AND AUTOMATION*. EOLSS Publications: 2009; Vol. II.

(3) ZoBell, C. E., Studies on Redox Potential of Marine Sediments1. *AAPG Bull.* **1946,** *30* (4), 477-513.

(4) Zimmermann, P.; Weltin, A.; Urban, G. A.; Kieninger, J., Active Potentiometry for Dissolved Oxygen Monitoring with Platinum Electrodes. *Sensors (Basel)* **2018,** *18* (8).

(5) Randles, J. E. B., Kinetics of rapid electrode reactions. *Discuss. Faraday Soc.* **1947,** *1* (0), 11-19.

(6) Newbery, E., Overvoltage and Transfer Resistance. *Proc. R. Soc. A* **1925,** *107* (743), 486-495.

(7) Lasia, A., *Electrochemical Impedance Spectroscopy and its Applications*. Springer: 2014.

(8) Macdonald, J. R.; Barlow, C. A., Theory of Double-Layer Differential Capacitance in Electrolytes. *J. Chem. Phys.* **1962,** *36* (11), 3062-3080.

(9) Westall, J. C., Reactions at the Oxide-Solution Interface: Chemical and Electrostatic Models. In *Geochemical Processes at Mineral Surfaces*, American Chemical Society: 1987; Vol. 323, pp 54-78.

(10) Bard, A. J.; Faulkner, L. R., *Electrochemical methods and applications*. Wiley-Interscience: New York; London, 2000.

(11) Sugimoto, Y.; Kitazumi, Y.; Tsujimura, S.; Shirai, O.; Yamamoto, M.; Kano, K., Electrostatic interaction between an enzyme and electrodes in the electric double layer examined in a view of direct electron transfer-type bioelectrocatalysis. *Biosens. Bioelectron.* **2015,** *63*, 138-144.

(12) Uchida, Y.; Kätelhön, E.; Compton, R. G., Cyclic voltammetry with non-triangular waveforms: Electrochemically irreversible and quasi-reversible systems. *J. Electroanal. Chem.* **2018,** *810*, 135-144.

(13) Elgrishi, N.; Rountree, K. J.; McCarthy, B. D.; Rountree, E. S.; Eisenhart, T. T.; Dempsey, J. L., A Practical Beginner's Guide to Cyclic Voltammetry. *J. Chem. Educ.* **2017,** *95* (2), 197-206.

(14) Adlam, D. J.; Woolley, D. E., A multiwell electrochemical biosensor for real-time monitoring of the behavioural changes of cells in vitro. *Sensors (Basel)* **2010,** *10* (4), 3732-3740.

(15) Charoenkitamorn, K.; Tue, P. T.; Kawai, K.; Chailapakul, O.; Takamura, Y., Electrochemical Immunoassay Using Open Circuit Potential Detection Labeled by Platinum Nanoparticles. *Sensors (Basel)* **2018,** *18* (2).

(16) Lu, S.; Guan, X.; Liu, C., Electricity-powered artificial root nodule. *Nat. Commun.* **2020,** *11* (1), 1505.

(17) Kolthoff, I. M.; Tomsicek, W. J., The Oxidation Potential of the System Potassium Ferrocyanide–Potassium Ferricyanide at Various Ionic Strengths. *J. Phys. Chem.* **1935,** *39* (7), 945-954.

(18) PalmSens, EmStat 3 and 3+ potentiostat. Author: Houten, NL, 2018.

(19) Gamry, Measuring Surface-related Currents using Digital Staircase Voltammetry. Author: Warminster, PA, 2015.

(20) Uchida, Y.; Kätelhön, E.; Compton, R. G., Cyclic voltammetry with non-triangular waveforms: Electrochemically reversible systems. *J. Electroanal. Chem.* **2017,** *801*, 381-387.

(21) Batchelor-McAuley, C.; Ellison, J.; Tschulik, K.; Hurst, P. L.; Boldt, R.; Compton, R. G., In situ nanoparticle sizing with zeptomole sensitivity. *Analyst* **2015,** *140* (15), 5048-54.

(22) Gross, P.-A.; Jaramillo, T.; Pruitt, B., Cyclic-Voltammetry-Based Solid-State Gas Sensor for Methane and Other VOC Detection. *Anal. Chem.* **2018,** *90* (10), 6102-6108.

(23) Kiam Heong, A.; Chong, G.; Yun, L., PID control system analysis, design, and technology. *IEEE Control Syst.* **2005,** *13* (4), 559-576.

(24) Guimera, X.; Moya, A.; Dorado, A. D.; Illa, X.; Villa, R.; Gabriel, D.; Gamisans, X.; Gabriel, G., A Minimally Invasive Microsensor Specially Designed for Simultaneous Dissolved Oxygen and pH Biofilm Profiling. *Sensors (Basel)* **2019,** *19* (21).

7 Appendix

The developed MATLAB® script was inserted here.

7.1 Codes for the Connection to Input/Output interface of device

```
if exist ('comm')
    disp('The device is connected, the iteration will be terminated')
    return;
end
clear;
PSSDK=LoadPSSDK();

deviceList = GetConnectedDevices(); %see function help for scanning bluetooth and serial devices
nDevices = deviceList.Count; %number of found devices
disp([newline num2str(nDevices) ' device(s) connected:'  newline]);
for i=0:nDevices-1
    disp([num2str(i+1) '. ' char(deviceList.Item(i).ToString())]);
end

%Prompt device to open a connection to
selectedDevice = 0;
while(selectedDevice < 1 || selectedDevice > nDevices)
    selectedDevice = input('\nSpecify the index of the device to open a connection to (or press
Control+C to cancel): \n \n');
    if(selectedDevice < 1 || selectedDevice > nDevices)
        disp(['Please enter a number between 1 and ' num2str(nDevices)]);
    end
end

%Open a connection to the device
selectedDevice = selectedDevice - 1;
deviceName = char(deviceList.Item(selectedDevice).ToString());
disp('');disp([newline 'Attempting to open a connection with ' deviceName]);
comm = OpenConnection(deviceList.Item(selectedDevice));
if(comm == false) %Check whether the connection was succesfully opened
    disp(['Error while opening a connection to ' deviceName '. Please check whether the specified
device' newline ' is a PalmSens or Emstat device, remove the usb and plug it back in, and try to
open the connection again' newline]);
    return;
end
disp(['Succesfully connected to ' deviceName]);
```

7.2 Codes for the Arbitrary Waveform

7.2.1 Calibration

```
method = NewMethod(8);
Interval=input('Get interval of each level, should be smaller than 0.1');
method.IntervalTime=Interval;
method.EquilibrationTime= 1;
timearray= [0:Interval:10];
%Customize the wavefore here, frequency
freq=2*pi;
%amplitude= E2
E2 = 0.4;
%Begin potential
E1 = 1.0;
%Customize potential array here
potentialarray= E1+ E2.*(sin(freq.*timearray));
for i=1:length(timearray)
level = PalmSens.Techniques.ELevel();
level.Duration = Interval;
level.Level = potentialarray(i); %Volts
method.Levels.Add(level);
end
mSetting=exist('m','var'); %Check measurement class
if mSetting == 0
    m=Measurement(comm);
    disp([newline 'Measurement class initialized.'])
    m.dispInCommandWindow=true;
    m.dispInPlot=true;
else
    disp('Measurement class is in place')
end
m.New(method);
tic;
while(m.inMeasurement)
    pause(0.01);
end
Time=toc;
Error=Time/i-Interval;
%Proceed the data acquisition after both processes stop at the same time
```

7.2.2 Arbitrary Waveform

```
% Run Calibration before running this file, if not it will fail
loadSetting=exist('selpath','var');
if loadSetting==0
    selpath = uigetdir(path,'Select folder to save');
else
    prompt=input('Need to Change folder setting? Y/N \n','s');
    if prompt == 'Y' || 'y'
        selpath = uigetdir(path,'Select folder to save');
    end
end
while selpath==0
    disp('No path is defined. Please define a new path.')
    selpath = uigetdir(path,'Select folder to save');
end
clear loadSetting
clear prompt
disp('Define filename');
filename=uiputfile('*.csv');
mSetting=exist('m','var'); %Check measurement class
if mSetting == 0
    m=Measurement(comm);
    disp([newline 'Measurement class initialized.'])
    m.dispInCommandWindow=true;
    m.dispInPlot=true;
else
    disp('Measurement class is in place')
end
method = NewMethod(8);
method.IntervalTime=Interval;
method.EquilibrationTime= 1;
Endtime=input('Please define EndTime in s');
timearray= [0:Interval+Error:Endtime];
%Customize the waveform here, frequency
freq=2*pi;
%amplitude= E2
E2 = 0.4;
%Begin potential
E1 = 1.0;
%Customize potential array here
potentialarray= E1+ E2.*(sin(freq.*timearray)+sin(2.*freq.*timearray)+sin(3.*freq.*timearray));
for i=1:length(timearray)
level = PalmSens.Techniques.ELevel();
level.Duration = Interval;
level.Level = potentialarray(i); %Volts
method.Levels.Add(level);
end
disp('Press any key to start experiment');
pause();
m.New(method);
tic;
```

```
while(m.inMeasurement)
    pause(0.01);
end
Time=toc;
measurement = m.measurement;
data=[measurement.curves.xData' measurement.curves.yData'];
rawdata= [timearray' potentialarray' data(1:end-1,2)];
table=array2table(rawdata,'VariableNames',{'Time in s','Potential in V','Current in uA'});
writetable(table,fullfile(selpath,filename));
disp('Arbitrary Waveform completed');
```

7.2.3 CV

```
% Run Calibration.m before running this file, if not it will fail
loadSetting=exist('selpath','var');
if loadSetting==0
    selpath = uigetdir(path,'Select folder to save');
else
    prompt=input('Need to Change folder setting? Y/N \n','s');
    if prompt == 'Y' || 'y'
        selpath = uigetdir(path,'Select folder to save');
    end
end
while selpath==0
    disp('No path is defined. Please define a new path.')
    selpath = uigetdir(path,'Select folder to save');
end
clear loadSetting
clear prompt
mSetting=exist('m','var'); %Check measurement class
if mSetting == 0
    m=Measurement(comm);
    disp([newline 'Measurement class initialized.'])
    m.dispInCommandWindow=true;
    m.dispInPlot=true;
else
    disp('Measurement class is in place')
end
method = NewMethod(8);
method.IntervalTime = Interval;
answer=inputdlg('Please Input Scan Rate in mV/s','Input ScanRate');
scanrate=str2double(answer{1});
VoltageInterval = scanrate * 0.001*(Interval+Error);
answer=inputdlg({'Please input 1st Voltage in V','Please Input 2nd Voltage in V'},'Voltage
Setting');
FVoltage=str2doubl(answer{1});
SVoltage=str2double(answer{2});
if FVoltage>SVoltage
    VoltageInterval=-1*VoltageInterval;
end
array1 = [FVoltage:VoltageInterval:SVoltage];
```

```
array2 = [SVoltage:(-1*VoltageInterval):FVoltage];
Potentialarray = [array1 array2];
for i=1:length(Potentialarray)
    level = PalmSens.Techniques.ELevel();
    level.Duration = Interval;
    level.Level = Potentialarray(i); %Volts
    method.Levels.Add(level);
end
m.New(method);
STime=datetime(clock);
while(m.inMeasurement)
    pause(0.01);
end
STime(2,1)= datetime(clock);
measurement = m.measurement;
data=[measurement.curves.xData' measurement.curves.yData'];
plotarray=zeros(length(Potentialarray)*method.nCycles,1);
for j=1:method.nCycles
        plotarray(length(Potentialarray)*(j-1)+1:length(Potentialarray)*j)= Potentialarray
(1:end);
end
Time=linspace(0,length(plotarray)*(Interval+Error),length(plotarray));
rawdata=[plotarray data(1:end-1,2) Time'];
table=array2table(rawdata);
table{1:2,end+1}=STime;
table.Properties.VariableNames={'Potential in V','Current in uA','Time in s','Starting and Ending
Time'};
filename=join([num2str(i),'_',num2str(scanrate),'mVperS']);
fullname=fullfile(selpath,filename);
save(join([fullname,'.mat']), 'table');
writetable(table,join([fullname,'.csv']));
disp('CV completed');
```

7.3 Codes for the Self Adjustable Electrolysis

7.3.1 Loops

```
% This file allow you to run Chronoamp and OCP for a specific time and
%collect all data
loadSetting=exist('selpath','var');
if loadSetting==0
    selpath = uigetdir(path,'Select folder to save');
else
    prompt=input('Need to Change folder setting? Y/N \n','s');
    if prompt == 'Y' || 'y'
        selpath = uigetdir(path,'Select folder to save');
    end
end
while selpath==0
    disp('No path is defined. Please define a new path.')
    selpath = uigetdir(path,'Select folder to save');
```

```
end
clear loadSetting
clear prompt
mSetting=exist('m','var'); %Check measurement class
if mSetting == 0
    m=Measurement(comm);
    disp([newline 'Measurement class initialized.'])
    m.dispInCommandWindow=true;
    m.dispInPlot=true;
else
    disp('Measurement class is in place')
end
Chronoamp=NewMethod(7);
OCP=NewMethod(12);
OCP.IntervalTime=0.01;
answer=inputdlg({'Please Input Begining Potential in V','Interval Time','Run Time'},'Chronoamp
Setting',[1 35],{num2str(Chronoamp.Potential) num2str(Chronoamp.IntervalTime)
num2str(Chronoamp.RunTime)});
Chronoamp.Potential=str2double(answer{1});
Chronoamp.IntervalTime=str2double(answer{2});
Chronoamp.RunTime=str2double(answer);
answer=inputdlg('Time for OCP','OCP Setting',[1 40],{num2str(OCP.RunTime)});
OCP.RunTime=str2double(answer{1});
answer=inputdlg('Total RunTime in second','Define TotalRunTime');
while str2double(answer{1})<(Chronoamp.RunTime+OCP.RunTime)
    disp('Run Time is smaller than a cycle, Please reinput Time');
    answer=inputdlg('Total RunTime in second','Define TotalRunTime');
end
Cycle = ceil(str2double(answer{1})/(Chronoamp.RunTime+OCP.RunTime));
answer=inputdlg({'Please Input the desire OCP','Kp setting'});
DesiredOCP=str2double(answer{1});
Kp=str2double(answer{2});
disp('The name of the file will be displayed as xxxx_1_Chronoamp');
answer=inputdlg('Get experiment name (xxxx)');
trial=answer{1};
VoltageProfile=zeros(Cycle,4);
disp('Press Any Key to Run');
pause();
for i=1:Cycle
    VoltageProfile(i,1)=Chronoamp.Potential;
    m.New(Chronoamp);
    STime=datetime(clock);
    while(m.inMeasurement)
        pause(0.01);
    end
    STime(2,1)= datetime(clock);
    measurement = m.measurement;
    table=array2table([measurement.curves.xData' measurement.curves.yData']);
    table{:,3}=NaT;
    table{1:2,3}=STime;
    table{:,4}=0;
    table{1,4}=Chronoamp.Potential;
    table.Properties.VariableNames={'Time in S','Current in uA','Starting and Ending
```

```
Time','Potential'};
    filename=join([trial,'_',num2str(i),'_','Chronoamp']);
    save(fullfile(selpath,join([filename,'.mat'])), 'table');
    writetable(table,fullfile(selpath,join([filename,'.csv'])));
    clear table
    m.New(OCP);
    STime=datetime(clock);
    while(m.inMeasurement)
        pause(0.01);
    end
    STime(2,1)= datetime(clock);
    measurement = m.measurement;
    table=array2table([measurement.curves.xData' measurement.curves.yData']);
    table{:,3}=NaT;
    table{1:2,3}=STime;
    table.Properties.VariableNames={'Time in S','OCP in V','Starting and Ending Time'};
    filename=join([trial,'_',num2str(i),'_','OCP']);
    save(fullfile(selpath,join([filename,'.mat'])), 'table');
    writetable(table,fullfile(selpath,join([filename,'.csv'])));
    clear table
    firstOCP=measurement.curves.yData(1);
    disp(join(['The OCP for this cycle is ',num2str(firstOCP),' V.']));
    error=firstOCP-DesiredOCP;
    correction=error*Kp;
    Chronoamp.Potential=Chronoamp.Potential-correction;
    disp(join(['The potential for the next cycle is',num2str(Chronoamp.Potential),' V.']));
    VoltageProfile(i,2)=error;
    VoltageProfile(i,3)=correction;
    VoltageProfile(i,4)=firstOCP;
end
VoltageProfile=array2table(VoltageProfile,'VariableNames',{'Voltage applied(V)','Error of
OCP(V)','Correction of applied Voltage(V)','OCP in V'});
VSetting=exist(fullfile(selpath,'Voltage.csv'));
if VSetting == 0
    writetable(VoltageProfile,fullfile(selpath,'Voltage.csv'));
else
    filename=uiputfile('*.csv');
    writetable(VoltageProfile,fullfile(selpath,filename));
end
disp('Loops completed')
```

7.3.2 Infinite loops with break condition

```
%This file will run Chronoamp and OCP until the error is
%less than 0.005V from Desired OCP
%A pause at the end of each loop was added to refill medium
%Voltage profile will be saved under Voltage.csv
loadSetting=exist('selpath','var');
if loadSetting==0
    selpath = uigetdir(path,'Select folder to save');
else
```

```
    prompt=input('Need to Change folder setting? Y/N \n','s');
    if prompt == 'Y' || 'y'
        selpath = uigetdir(path,'Select folder to save');
    end
end
while selpath==0
    disp('No path is defined. Please define a new path.')
    selpath = uigetdir(path,'Select folder to save');
end
clear loadSetting
clear prompt
mSetting=exist('m','var'); %Check measurement class
if mSetting == 0
    m=Measurement(comm);
    disp([newline 'Measurement class initialized.'])
    m.dispInCommandWindow=true;
    m.dispInPlot=true;
else
    disp('Measurement class is in place')
end
Chronoamp=NewMethod(7);
OCP=NewMethod(12);
OCP.IntervalTime=0.01;
answer=inputdlg({'Please Input Begining Potential in V','Interval Time','Run Time'},'Chronoamp
Setting',[1 35],{num2str(Chronoamp.Potential) num2str(Chronoamp.IntervalTime)
num2str(Chronoamp.RunTime)});
Chronoamp.Potential=str2double(answer{1});
Chronoamp.IntervalTime=str2double(answer{2});
Chronoamp.RunTime=str2double(answer);
answer=inputdlg('Time for OCP','OCP Setting',[1 40],{num2str(OCP.RunTime)});
OCP.RunTime=str2double(answer{1});
answer=inputdlg({'Please Input the desire OCP','Kp setting'});
DesiredOCP=str2double(answer{1});
Kp=str2double(answer{2});
disp('The name of the file will be displayed as xxxx_01_Chronoamp');
answer=inputdlg('Get experiment name (xxxx)');
trial=answer{1};
VoltageProfile=zeros(1,4);
i=0;
disp('Press Any Key to Run');
pause();
while true
    i=i+1;
    VoltageProfile(i,1)=Chronoamp.Potential;
    m.New(Chronoamp);
    STime=datetime(clock);
    while(m.inMeasurement)
        pause(0.01);
    end
    STime(2,1)= datetime(clock);
    measurement = m.measurement;
    table=array2table([measurement.curves.xData' measurement.curves.yData']);
    table{:,3}=NaT;
```

```
    table{1:2,3}=STime;
    table{:,4}=0;
    table{1,4}=Chronoamp.Potential;
    table.Properties.VariableNames={'Time in S','Current in uA','Starting and Ending
Time','Potential'};
    filename=join([trial,'_',num2str(i),'_','Chronoamp']);
    save(fullfile(selpath,join([filename,'.mat'])), 'table');
    writetable(table,fullfile(selpath,join([filename,'.csv'])));
    clear table
    m.New(OCP);
    STime=datetime(clock);
    while(m.inMeasurement)
        pause(0.01);
    end
    STime(2,1)= datetime(clock);
    measurement = m.measurement;
    table=array2table([measurement.curves.xData' measurement.curves.yData']);
    table{:,3}=NaT;
    table{1:2,3}=STime;
    table.Properties.VariableNames={'Time in S','OCP in V','Starting and Ending Time'};
    filename=join([trial,'_',num2str(i),'_','OCP']);
    save(fullfile(selpath,join([filename,'.mat'])), 'table');
    writetable(table,fullfile(selpath,join([filename,'.csv'])));
    clear table
    firstOCP=measurement.curves.yData(1);
    disp(join(['The OCP for this cycle is ',num2str(firstOCP),' V.']));
    error=firstOCP-DesiredOCP;
    correction=error*Kp;
    Chronoamp.Potential=Chronoamp.Potential-correction;
    disp(join(['The potential for the next cycle is',num2str(Chronoamp.Potential),' V.']));
    VoltageProfile(i,2)=error;
    VoltageProfile(i,3)=correction;
    VoltageProfile(i,4)=firstOCP;
    if abs(error) < 0.005
        break;
    end
    disp('Press Any Key to Continue')
    pause();
end
VoltageProfile=array2table(VoltageProfile,'VariableNames',{'Voltage applied(V)','Error of
OCP(V)','Correction of applied Voltage(V)','OCP in V'});
VSetting=exist(fullfile(selpath,'Voltage.csv'));
if VSetting == 0
    writetable(VoltageProfile,fullfile(selpath,'Voltage.csv'));
else
    filename=uiputfile('*.csv');
    writetable(VoltageProfile,fullfile(selpath,filename));
end
disp('Infinite Loop completed')
```

7.4 Codes for Disconnection

```
f=warndlg('You are about to erase all data!!','Warning');
prompt=input('Are you sure to Disconnect the erase the workspace? Make sure you save all the
file!! Y/N \n','s');
if prompt=='Y' || prompt=='y'
    delete (m);
    clear m;
    comm.Disconnect();
    delete(comm); %Deleting this object removes its references to the PalmSens library and frees
up resources
    clear comm
    clear nDevices
    clear selectedDevice
    clear i
    clear deviceList
    clear deviceName
    clear all
end
```